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ANTIMICROBIAL RESISTANCE AND ALTERNATIVE TREATMENTS: A COMPREHENSIVE REVIEW

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ABSTRACT

The escalating global threat of antimicrobial resistance (AMR) jeopardizes human health and healthcare systems worldwide. This review delves into the intricacies of AMR, its impact on health, and the role of livestock and aquaculture in driving its emergence. The misuse of antibiotics in human and veterinary medicine has expedited AMR development. Livestock and aquaculture use antimicrobials as growth promoters, creating a breeding ground for AMR due to constant sublethal antibiotic exposure.

AMR's prevalence discourages new antibiotic development, exacerbated by microorganisms' adaptability through genetic mutations and horizontal gene transfer. Counteracting AMR involves exploring alternative treatments. Bacteriophage therapy, predatory bacteria, and probiotics show promise in recent studies. Antimicrobial peptides, aptamers, antibody-antibiotic conjugates, and gene-editing tools like CRISPR-Cas are under investigation. Nanomaterials, vaccines, and plant-derived compounds also exhibit efficacy.

Although these alternatives hold potential, they've not entirely addressed the antibiotic gap. They do, however, complement existing therapies, maximizing impact. In summary, AMR endangers human life, with aquaculture and livestock accelerating its rise. Microorganisms' adaptability hampers antibiotic development, necessitating alternative strategies. While promising, further research is crucial to fully exploit these methods, safeguarding human health from the AMR menace.

KEYWORDS: Antimicrobial Resistance, Antibiotics, Antimicrobial Resistant Genes, Aquaculture, Phage Therapy

INTRODUCTION

Antimicrobial resistance (AMR) is a pressing global health concern that threatens the efficacy of conventional antibiotics and poses a significant risk to human life. The emergence and spread of resistant microorganisms have been attributed to various factors, including the misuse and overuse of antibiotics in human medicine, as well as the widespread use of these agents in agriculture, particularly in aquaculture and livestock farming practices(Cabello, 2006; Llor & Bjerrum, 2014). The interconnectedness of human and animal health highlights the urgent need for comprehensive strategies to address AMR effectively.

Aquaculture and livestock farming have become major contributors to the dissemination of AMR. Antibiotics are routinely administered to promote growth and prevent infections in these industries, resulting in a selective pressure that favors the survival and proliferation of resistant bacteria(Cabello, 2006). The overuse of antibiotics in these settings has been linked to the transfer of resistance genes to human pathogens through food consumption, environmental contamination, and direct contact with animals(Kruse & Sørum, 1994; Le Roux & Blokesch, 2018; J. L. Martínez, 2012). Such transmission pathways have facilitated the emergence of multidrug-resistant bacteria, reducing the available treatment options for infectious diseases in both animals and humans.

One of the concerning aspects of AMR is the intrinsic ability of microorganisms to rapidly develop resistance mechanisms. Bacteria possess an impressive capacity for genetic adaptation and can acquire resistance genes through mutation or horizontal gene transfer(Noster et al., 2021). This genetic plasticity allows bacteria to quickly develop resistance to antibiotics, rendering these drugs ineffective in combating infections. The evolution of resistance is further exacerbated by the widespread and often inappropriate use of antibiotics, as well as inadequate infection prevention and control measures in healthcare settings(Murray et al., 2022). The high rate of resistance development, coupled with the relatively slow pace of new antibiotic discovery, has discouraged pharmaceutical companies from investing in antibiotic research and development(Doern, 2014).

To address the challenges posed by AMR, alternative treatments and techniques have emerged as potential substitutes for conventional antibiotics. These alternatives encompass a range of strategies, including phage therapy, predatory bacteria, probiotic bacteria treatment, antimicrobial peptides, oligonucleotide aptamers, antibody-antibiotic conjugates, antisense/antigene oligonucleotides, artificial restriction nucleases (e.g., zinc finger nucleases, TALENs, CRISPR-Cas), efflux pump inhibitors, nanomaterials, vaccine development, and phytochemicals. These approaches target different aspects of microbial physiology and offer potential solutions to combat AMR and reduce the reliance on traditional antibiotics. However, while they show promise, challenges such as limited clinical trials and regulatory approval hinder their widespread implementation as alternatives to antibiotics.

Group of humans most vulnerable of AMR infection

- **Cancer patient:** The growth of tumors, their malignancy, or other associated disorders such as sepsis, multiphasic host response to a pathogen that endogenous factors can dramatically increase can all-cause death in cancer patients. Infections are common in cancer patients, and effective antibiotics are needed to prevent and cure bacterial infections. Antibiotic failure in cancer patients raises the risk of sepsis, sepsis-related death, and healthcare expenses. Antibiotic resistance threatens to destroy much of the hard-won progress against cancer; thus, optimizing current medications and discovering new antibiotics is critically important to protect patients with cancer from antibiotic-resistant infections. (Lee et al., 2013; Vázquez-López et al., 2019; Williams et al., 2004)
- **Organ and bone marrow transplant:** In hematopoietic stem cell transplantation (HSCT) patients, bacterial infections are the most common cause of infectious complications, especially in the early post-transplant period. Infectious problems are linked to a high morbidity and mortality rate in HSCT recipients(Alp & Akova, 2017). With successful results, hematopoietic stem cell transplantation (HSCT) has become the therapeutic option for many hematological cancers and diseases (Mackall et al., 2009). In febrile neutropenic HSCT recipients, introducing broad-spectrum empirical antibiotic therapy reduces mortality. On either side, using such medication risks selecting resistant pathogens (Alp & Akova, 2013). Fluoroquinolone & quinolone prophylaxis with empirical carbapenem use on patients in hematology settings led to emerging antimicrobial resistance like fluoroquinolone resistance, methicillin-resistant Staphylococcus aureus (MRSA), multidrug-resistant (MDR) *Escherichia coli, Pseudomonas aeruginosa bacteremia, Clostridium difficile* infections, and carbapenem-resistant bacterial infections respectively (Castagnola et al., 2005; Kern et al., 2005; Rangaraj et al., 2010).
- **Dialysis for end-stage Renal disease:** Bacterial infections are a prominent cause of morbidity and death in people with End-stage renal disease, including bloodstream infections coupled with hemodialysis and peritonitis associated with peritoneal dialysis. Furthermore, bacteria that cause dialysis-related illnesses frequently include AMR (Antimicrobial Resistance) determinants *(ANZDATA 40th Annual Report 2017 (Data to 2016)*, n.d.). Pathogens linked to hemodialysis-associated bloodstream infections have a wide

distribution range and antibiotic resistance patterns across geographic locations. Methicillin resistance rates in S. aureus bloodstream infection isolates have ranged from 0% in Danish research to 100% in a single-center Algerian investigation. (Skov Dalgaard et al., 2015). Antibiotic resistance is a rising concern among peritoneal dialysis-associated infections, just as it is among hemodialysis-associated infections, and the frequency of antimicrobial resistance among these pathogens varies geographically. Analysis of cases of dialysis-associated peritonitis reported at a single center in northern India between 2002 and 2011 revealed significant resistance rates across different pathogens. A total of 303 peritonitis episodes with an overall incidence of 0.41 per patient-year were recorded, coagulase-negative Staphylococcus spp. (CONS) was the most common isolate present in the record. Patients with peritonitis caused by vancomycin-resistant enterococci, ESBL- and MBL-producing bacteria had a much higher mortality rate(Prasad et al., 2014). After kidney transplantation, infection is a major cause of morbidity and death. The most prevalent infections in the first month following transplantation are healthcare-associated infections, including surgery-related and donor-derived infections. The changing global epidemiology of infections, rising antibiotic resistance, unsatisfactory tests for microbiologic screening of organ donors, and virus-associated cancers are all major challenges. (Fishman, 2017). Due to frequent exposure to antibiotics and healthcare settings, post-surgical anatomic anomalies such as vesicoureteral reflux, ureterovesical junction stenosis, or neurogenic bladder may predispose transplant patients to recurrent UTIs and increase the chance of acquiring or developing AMR(Ariza-Heredia et al., 2013). Depending on the infection, organism, and locale, the incidence of multidrug resistance among bacteria isolated from kidney transplant recipients varies substantially, ranging from 8% to 46% (Aguado et al., 2018; Bodro et al., 2013)

- **Rheumatoid arthritis:** Disturbances in the human microbiota caused by antibiotics have been related to the development of chronic autoimmune diseases(Sultan et al., 2019). Rheumatoid arthritis (R.A.) is a chronic autoimmune inflammatory disease characterized by the generation of autoimmune antibodies that cause bone joint damage and related R.A. pathology. Antibodies to citrullinated peptide antigens (ACPA) are generated in response to bacterial components resembling host cell receptors, such as those produced by *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans.* (Konig et al., 2016; Smolen et al., 2018). The correlation between antibiotics and R.A. patients is conducted by studying the history of prescription antibiotics. For example, in a nested-case study, R.A. cases received more antibiotic prescriptions than controls. Still, over 80% in each group had received a prescription ten years before the index date. Penicillins were the most regularly given antibiotics (72% of participants had at least one prescription before the index data), followed by macrolides (33%) and trimethoprim (31%), cephalosporins (25%), and tetracyclines and quinolones (25%). (both 13%). After controlling for potential confounding variables, the likelihood of having been exposed to antibiotics was considerably greater in R.A. patients than in control(Sultan et al., 2019)
- **Complex surgery:** A surgical site infection concerns heart bypass patients, orthopedic implants, and other major procedures (SSI). These infections can make it more difficult to recuperate following surgery by causing additional sickness, stress, cost, and death. Antibiotics are used before certain, but not all, procedures to help avoid infections. Surgical site infections are associated with high morbidity, mortality, and healthcare expenditures. The advent of multidrug-resistant bacteria in hospitals has become a worldwide concern for surgeons who treat healthcare-associated infections (Yehouenou et al., 2020). Antibiotic resistance mechanisms acquired by pathogenic bacterial strains have highlighted issues in managing SSIS worldwide. Methicillin-resistant Staphylococcus aureus (MRSA), Enterobacteriaceae that produce extended-spectrum beta-lactamases (ESBLs), and the presence of polymicrobial flora and fungus have all exacerbated these problems. Escherichia coli (15.9%), Staphylococcus aureus (14.8%), Enterococcus spp. (10.2%), Pseudomonas aeruginosa (8.9%) and Klebsiella spp. (8.9%) Among the most often identified microorganisms From healthcare-associated infections (HAIs) (8.9%). S. aureus was resistant to methicillin in 28.2% of cases, while enterococci were resistant to vancomycin in 14.2% of cases(Friedrich, 2019; Salmanov et al., 2019). Patients who undergo cardiac surgery appear to be at increased risk for developing Nosocomial infections (N.I.s).In a study, the most common NI-causing bacteria were *E.coli and S.aureus.* ESBL-producing bacteria were found in 14.28-71.42 percent of *Enterobacteriaceae*, whereas MRSA was found in 54.2% of *S.aureus strains* (Davoudi et al., 2016)

Alternative to antibiotics

Because antibiotic resistance strategies have solely focused on controlling the resistance process, there is an urgent need to discover new ways to tackle the problem. Numerous ways have both benefits and drawbacks when used as an alternative to antibiotics; these alternative therapies might be used individually or in combination with antibiotics to overcome resistance. Some may not be as effective as antibiotics, but they may buy us time until we can develop new antibiotics.

- **Phage therapy**

Bacteriophages are bacteria-infecting viruses that destroy bacteria without harming human or animal cells. As a result, it is believed that they can be used to treat bacterial infections alone or in collaboration with antibiotics; they are easy to discover, only small doses would be sufficient enough, and they are completely harmless to the environment as well(Domingo-Calap & Delgado-Martínez, 2018).

In several studies, the bacteriophage cocktails have worked better than antibiotics by targeting pathogenic bacteria and reducing the risk of pathogenic invasion while preventing non-targeted commensal microbiota of mice models and human cells(Jakobsen et al., 2022; Lamy-Besnier et al., 2021; Moye et al., 2019). Moreover, the administration of phages could overcome several problems related to the administration of antibiotics, such as the emergence of secondary infection, disperse biofilms, attacking gut microbiome or other beneficial microorganisms, and prevention of the emergence of antimicrobial resistance(Domingo-Calap & Delgado-Martínez, 2018; Gutiérrez et al., 2016). However, injecting bacteriophages directly into the human body has significant drawbacks; hence, the European Medicine Agency or the Food and Drug Administration has not yet approved human use. Isolation of bacteriophage is easy, but identification of therapeutic phage is complicated because phages are bacterial specific and do not work on every bacterial strain as a therapy. Hence immunological studies have to be conducted for each bacterial strain while maintaining stability which could be time-consuming and demotivating enough for pharmaceutical companies to prepare relevant therapeutic agents(Cisek et al., 2017). The phage genome must be sequenced and must not contain integrase genes, antibiotic resistance genes (ARG), genes for phage-encoded toxins, or other bacterial virulence factors, as in the lysogenic type. Finally, issues concerning the formulation and stability of pharmacological formulations for clinical usage remain unsolved(Vandenheuvel et al., 2015).

Moreover, bacterial strains could become resistant to phage therapy too by preventing themselves from a viral infection by following defense strategies, for example, concealing, modification, or loss of receptors, production of molecules that hinder phage attachment to the bacterial pathogen, activation of mechanisms to prevent phage DNA injection into the cell, and suppression of phage replication and release(Seed, 2015). The use of phage cocktails, the delivery of a greater initial phage inoculum, or the combination with antibiotics can help decrease the development of bacterial resistance to bacteriophages(Yang et al., 2020) primary role of innate immune cells is to recognize and eradicate foreign material. Leukocytes may bind phages and eradicate the therapeutic bacteriophages assuming foreign body by triggering an immune response(Jończyk-Matysiak et al., 2017). Hence, the clinical significance of phage treatment is still unclear(Krut & Bekeredjian-Ding, 2018).

- **Predatory Bacteria**

Bacteria that feed on other bacteria are known as predatory bacteria. In the absence of their prey, certain predatory bacteria can switch from predation to feeding on other substrates, while others rely on their prey for sustenance; these bacteria rupture the membrane of their prey, multiply or grow inside and kill(Bratanis et al., 2020). Endobiotic predators (adhere from outside, perforate periplasmic membrane by releasing hydrolytic enzyme) like *B. bacteriovorus* (Sockett, 2009) and certain individual predators with an epibiotic approach (solitary hunters) like *M. aeruginosavorus* (Kadouri et al., 2013)are gaining attention as potential treatments for antibiotic-resistant diseases. Using such bacteria as biocontrol agents to prevent undesirable pathogenic bacteria from water bodies or aquaculture farms could be cheap, cheap, and less harmful (Kandel et al., 2014; V. Martínez et al., 2016). Nevertheless, such bacterial predator agents could lead to bacterial resistance and incomplete eradication of the prey. However, repeated culture appears to restore the prey's sensitivity to predation (Sockett, R., and Lambert, C. (2004), n.d.).

- **Probiotic Bacteria**

Microorganisms that live in symbiosis with the human host are known as probiotics. Probiotics may alter biological activities and provide health advantages when consumed sufficiently (Reid, 2006). The gut microbiota could be disrupted by prolonged use of antibiotics to treat infection; this could lead to bacterial invasion in particular infected organs and spread to other organs, too(Khan et al., 2016). Hence, inoculation of live cells compatible with human microflora and also resistant to such antibiotics could be the solution to altering gut flora; for example, in a study by(Lokesh et al., 2019), probiotic organisms including *Bifidobacterium adolescentis, Bifidobacterium animalis, and Bifidobacterium longum* demonstrate resistance to antibiotic and flourish luxuriantly in the presence of the other three first-line antitubercular medicines. Probiotics have been shown to reduce the number of antibiotic resistance genes in the gut of colonization-permissive patients (Montassier et al., 2021).

Probiotics have vast use in industrial and aquaculture sectors too to provide better nutrient uptake in food animals, enhancement of immune response, maintain water quality and growth and prevent from spreading of antimicrobial resistance genes in the produce (Kumar et al., 2016; Martínez Cruz et al., 2012), according to (Verschuere et al., 2000) The use of probiotics to implement microbial management methods in hatcheries may be advantageous to the cultures. A robust, nonpathogenic, and diversified adherent microbiota on the eggs would most likely act as a barrier to pathogen colony development in fish eggs. However, on the other hand, antibiotic resistance determinants carried on mobile genetic components, including tetracycline resistance genes, Plasmid-associated antibiotic resistance, presence of transferable antibiotic resistance genes probiotics is a real cause for concern since that might make it difficult to use probiotics due to the risk of resistance spreading to hazardous microbes living in the same niche(Sharma et al., 2014).

- **The Antimicrobial Peptides (AMPs)**

Antimicrobial peptides (AMPs) are cationic (positively charged) and amphiphilic (hydrophilic and hydrophobic) -helical peptide molecules that play a role in host defence. These peptides generally have less than 100 amino acids (Jenssen et al., 2006). These cationic AMPs can bind to and interact with negatively charged bacterial cell membranes, causing potential electrochemical changes on the membranes, cell membrane degradation, and the penetration of bigger molecules like proteins, damaging cell shape, and membranes, causing cell death (Moravej et al., 2018).AMPs could be useful in combination with antibiotics for therapies (Wu et al., 2017) or as an alternative to antibiotics to prevent antibiotic resistance(Manniello et al., 2021). However, alteration of bio-film molecules, production of protective material, and up-regulation or removal of particular proteins can all be used to establish a resistance to AMPs. Bacterial resistance to AMPs frequently involves biophysical and biochemical alterations in pathogens due to the general processes of AMP binding and activation(Abdi et al., 2019;Weiss, 2015). Other disadvantages of using AMPs include a high rate of failure in clinical studies and clinical concerns about the levels of toxicity (Kollef et al., 2006; Moravej et al., 2018; van der Worp et al., 2010).

- **Oligonucleotide Aptamers**

Oligonucleotide aptamers are single-stranded DNAs or RNAs with target-selective high-affinity properties used as nucleic acid-based affinity ligands to replace monoclonal antibodies(Afrasiabi et al., 2020). Aptamers for particular ligands are frequently found using the approach of systematic evolution of ligands by exponential enrichment (SELEX). SELEX provides more control over binding conditions and selection under non-physiological settings(Famulok & Mayer, 2014). As a result, high-affinity, chemically stable aptamer probes can be constructed for targets that are very toxic or do not trigger an immune response in vivo, even though some aptamers are present in nature as ligand-binding sites of unique RNA structures known as riboswitches (McKeague et al., 2015). Aptamer-based systems have been discovered to be effective tools in the treatment of microbial infections due to their promising anti-biofilm and antimicrobial activities; they can reduce or inhibit the effects of bacterial toxins, inhibit pathogen invasion into immune cells, and can be used in drug delivery systems(Afrasiabi et al., 2020; Yoon & Rossi, 2018). Aptamers can be combined with siRNAs, fluorophores, radioisotopes, electrochemical systems, and different nanoparticles for a better outcome. E.g., when mesoporous silica nanoparticles (MSNs) were employed for bacteria-targeted delivery, aptamer-gates generated a significant decrease in the minimum inhibitory concentration (MIC) values of vancomycin for Staphylococcus aureus(Kadioglu et al., 2015). When polyclonal aptamers were linked to human C1qrs protein and streptavidin-C1qrs complex either with or without a linker, a substantial reduction in colony counts was seen when applied to E. coli O111:B4 and K12 strains throughout a series of 10 dilutions in the presence of human serum(Bruno et al., 2008). Aptamer-based therapeutics typically employ one of three strategies: (1) an aptamer acts as an antagonist, preventing disease-associated targets, such as receptor-ligand interactions; (2) an aptamer acts as an agonist, activating the function of target receptors; or (3) a cell type-specific aptamer acts as a carrier, delivering other therapeutic agents to the target cells or tissue. More research is required to overcome obstacles such as short in vivo half-lives, rapid excretion via renal filtration (i.e., less circulation time) and continuous or repeated administration of aptamer therapeutics, toxicities such as polyanionic effects, unexpected tissue accumulation, intensive chemical modification or conjugation, and nonspecific immune activation could be generated(Zhou & Rossi, 2017)

- **Antibody-antibiotic conjugates (AACs).**

Methicillin-resistant Staphylococcus aureus (MRSA) traces were found in macrophages even after treating the MRSA strain with antibiotics like vancomycin, which might be enough to infect other cells and tissues. A team at Genentech developed antibody-antibiotic conjugates, which are comparable to cancer treatment. AACs are antibodies obtained specifically from B cells of patients who had survived s.aureus infection, which is then linked to a highly potent antibiotic that can function within the acidic environment of phagocytic lysosomes, as well as a protease cleavable valine-citrulline linker that allows antibiotic release inside phagosomes. *ACCs* surpassed vancomycin because they destroyed the MRSA inside the host cell after it was activated(Koenig & Pillow, 2019; Lehar et al., 2015; Linghu et al., 2018). This approach could help fight antibiotic-resistant microorganisms(Kajihara et al., 2021; Motley & Fries, 2017). Proper assessment of pharmacokinetics/physicochemical dynamic, hydrophobic interaction, toxicity level, stability, antigen-binding stoichiometry, and aggregation formation might be a big problem with AACs due to their restricted therapeutic index. Although AAC development is difficult because of the intricacy of the three components (antibody, antibiotic, and linker), several effective examples are now being researched in clinical studies(Cavaco et al., 2022).

- **Antisense/Antigene oligonucleotides**

Antisense oligonucleotides used to prevent the production of proteins in bacterial cells be useful in treating bacterial infections. Antisense antimicrobials are short, single-stranded oligomers that bind to particular, complementary RNA in a target organism and imitate the structure of DNA or RNA. Antisense therapeutics attach to complementary mRNA in bacteria and either block the initiation of translation by sterically blocking the 30S ribosomal subunit or by accelerating the activation of RNase, leading to the breakdown of the targeted mRNA(Dias & Stein, 2002). Numerous chemical structures might be employed as a potential molecular tool that operates on the concept of antisense technology, such as locked nucleic acids (LNA)(Orum & Wengel, 2001), bridging nucleic acids (BNA)(Linse et al., 2015), phosphorothioates (S-DNA)(Bertram et al., 1995), phosphorodiamidate morpholino-oligomers (PMO)(Sully et al., 2017), and peptide nucleic acids (PNA)(Yavari et al., 2021). This antisense oligonucleotide can be co-administered with AMPs or antibiotics themselves for better results(Hansen et al., 2016)

The concept of employing antisense therapies as bacterial resistance modulators is generally applicable and might be utilized to overcome resistance in any pathogenic species. Moreover, unlike drugs targeting critical genes, it may target just antibiotic-resistant bacteria, thus reducing disturbance of the commensal bacteria, especially if the antisense facilitates the co-administration of a narrow-spectrum drug. Toxicity is also expected to be low as antibiotic resistance genes have little in common with human genes, and people are constantly exposed to bacterial nucleic acids. However, many challenges need to be addressed before these breakthrough technologies can be used to minimize the burden of antibiotic resistance for the benefit of patients(Woodford et al., 2009).

- **Artificial restriction nucleases (zinc finger nuclease (ZFN), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat – CRISPR-associated (CRISPR-Cas) systems)**

In contrast to several natural restriction enzymes obtained from bacteria and archaea, artificial restriction enzymes that cleave DNA at any specified sequence can now be synthesized. One of the goals of this technique is to target antimicrobial resistance genes and potentially pathogenic or virulent genes in mobile DNA elements or the bacterial genome, knocking these genes out and inhibiting horizontal gene transfer between bacteria. Zinc finger nuclease ZFNs and Transcription activator-like effector nucleases (TALENs) are restriction nucleases designed and programmed to cleave particular DNA sequences and are also used for gene editing. These proteins are coupled to a nonspecific endonuclease of the type IIS FokI restriction enzyme, which gives ZFNs and TALENs nuclease activity(Guo et al., 2010; Kim et al., 1996). The zinc finger domain identifies a 3- to the 4-bp DNA sequence, whereas tandem domains may bind to a longer nucleotide sequence unique within a cell's genome. ZFNs are designed as a pair that detects two sequences around the site, one on the forward strand and the other on the reverse strand, to leave a specific location in the genome. The pair of FokI domains dimerize and cleave the DNA at the site upon binding the ZFNs on each side of the site, resulting in a double-strand break with five ′ overhangs. This double-strand breakage is repaired by either nonhomologous end joining (which can occur at any stage of the cell cycle), or homology-directed repair (which often happens in late S or G2 phases when a sister chromatid is present to act as a repair template) and this can be accomplished by employing an exogenous double-stranded DNA vector as a repair template(Urnov et al., 2010). In recent years clustered regularly interspaced short palindromic repeat – CRISPR-associated (CRISPR-Cas) systems is a breakthrough technology used in molecular and genetic engineering research as an important tool for gene editing in eukaryotic cells(Ran et al., 2013). ZFN and TALEN are both artificial tools invented by humans, but CRISPR is a bacterial survival mechanism. ZFN and TALEN are both designed nucleases. CRISPR is composed of two kinds of RNA that work along with Cas proteins. RNA–DNA interactions govern DNA site recognition in the CRISPR-Cas9 system. which has numerous advantages over ZFNs and TALENs, including a straightforward design for any genomic target, easy prediction of off-target locations, and the ability to simultaneously change many genomic sites(Gaj et al., 2013). The capacity to discriminate between commensal and harmful bacterial species is one of this system's most dynamic and highly specialized essential aspects. Guide CRISPR-RNA could be designed to target pathogen-specific chromosomal and virulence genes, allowing this breakthrough approach to be employed against bacteria rather than protecting against invasions. For example, the CRISPR/Cas9 "pro-active" genetic system (Pro-AG) could be utilized to eradicate bacterial virulence components carried on virulence plasmids as well as resistance determinants in commensal bacteria(Valderrama et al., 2019). The main barrier to using CRISPR–Cas

systems as antibacterial agents is the lack of an effective and targeted delivery strategy. Although viral techniques are the most often used CRISPR/Cas9 delivery vehicle, they have significant limitations, including potential target effects and immunogenicity. Utilizing Nanocomplexes or attachment of potential peptides has been employed successfully in both bacteria and humans to deliver CRISPR–Cas(Gupta et al., 2021).

Artificial restriction nucleases technology might be used to eliminate resistance by targeted disruption of the resistance gene, such as when targeting ampicillin resistance using ZFN technology, which results in the inactivation of β -lactam production. As a result, artificial restriction nucleases technology might be used to reduce antibiotic resistance by building a ZFN, TALEN, or CRISPR archive against various ARGs. Recombinant phages producing ZFNs against various ARGs could be developed and discharged into hospital and metropolitan wastewater systems to address the issue of resistance at the environmental level(Shahbazi Dastjerdeh et al., 2016).

- **Efflux pump inhibitors (EPIs)**

Efflux pumps are proteinaceous transporters found in all cells' cytoplasmic membranes. Efflux pumps may move a range of harmful substances out of cells, including drugs, trace metals, organic contaminants, plant-produced compounds, quorum sensing signals, microbial metabolites, and neurotransmitters, through active efflux important aspect of xenobiotic metabolism. Certain efflux systems are drug-specific, while others could tolerate numerous drugs using small multidrug resistance (SMR) transporters(Sun et al., 2014). Bacterial efflux pumps are classified into five families based on their amino acid sequence, the energy source used to export their substrates, and the number of transmembrane spanning regions: the multidrug and toxic compound extrusion (MATE) family. The major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, the ATP (adenosine triphosphate)-binding cassette (ABC) superfamily, the resistance-nodulation-division (RND) family(Putman et al., 2000). The genetic components that encode efflux pumps may be encoded on chromosomes and plasmids, leading to intrinsic and acquired resistance(Li et al., 2015). Almost all antibiotics are sensitive to active efflux, and like many, their value has been reduced due to pathogen pump overproduction. Active drug efflux, particularly generated by RND pumps, plays a significant role in intrinsic and acquired resistance in Gram-negative bacteria. However, suppose such pumps can now be inhibited. In that case, a large range of therapeutic compounds which could modify or accelerate the action of the principal drugs may become available and restore the effectiveness of antibiotics(Blanco et al., 2016). EPIs have been demonstrated to suppress biofilm formation, which is essential in pathogenesis and necessitates the presence of RND pumps in many pathogenic bacteria(Soto, 2013).

One of the examples of possible efflux pump inhibitors used to combat efflux pump-related antimicrobial resistance is provided. PAβN was a broad-spectrum inhibitor of three major RND pumps in *P. aeruginosa*, MexAB-OprM, MexCD-OprJ, and MexEF-OprN, for fluoroquinolone efflux, as well as an inhibitor of the E. coli AcrAB-TolC pump(Lamers et al., 2013).

- **Nanomaterials (N.M.)**

Antibiotic-resistant bacterial infections caused by acquired resistance and biofilm formation demand the development of novel therapies(Beyth et al., 2015). Antibacterial treatments based on nanomaterials are promising. Nanomaterials can target biofilms because of their unique size and physical features (Makabenta et al., 2021). Nanomaterials block quorum sensing molecules and inhibit bacterial biofilms, inhibit DNA replication, penetrate the cell envelope by causing oxidative stress, and alter membrane permeability, resulting in cell lysis and adsorption damaging lipid and protein present on the bacterial cell surface(Hemeg, 2017). The effectiveness of these nanoparticles varies depending on their size, shape, and concentration. Furthermore, the atomic abundance on the surface of the particles influences the characteristics of such materials. Due to their specialized sites of action, small molecular antibiotics enable the emergence of resistant pathogens.

In contrast, antimicrobial nanomaterial physically damages the organism's cell membranes, preventing the generation of drug-resistant microorganisms(Beyth et al., 2015). Most effective nanoparticles are developed using expensive metals like silver and gold or metals such as copper, which require an additional coating to prevent corrosion(Dizaj et al., 2014); hence, treatment could be expensive. Organic Nanoparticles could crystallize after prolonged storage, accumulate in tissues, or give allergic reactions(Roma-Rodrigues et al., 2020). As a result, the use of nanomaterials as an antibacterial alternative is still debatable.

- **Vaccine development**

According to WHO, vaccines could limit the spread of antibiotic resistance. Vaccinating humans and animals is a highly successful approach to avoid infection and, as a result, the need for medicines. People sometimes take antibiotics needlessly when they have symptoms like fever or strep throat that a virus might cause; thus, vaccines against viruses like the flu play a key role(WHO,2016, n.d.).

Vaccines are routinely used to protect livestock from infection. Vaccines must be safe, effective, easy to use, and cost-effective to be extensively employed in food-producing animals. In one or more of these areas, many existing vaccinations fall short. Prioritize research to ensure that restricted public resources are allocated to areas with the greatest positive risk first, and private investments in vaccine development must compete with other investment possibilities(Hoelzer et al., 2018). High cost of production, rigorous administration procedures, development of vaccines for mass application, multiple booster vaccinations, and Regulatory restrictions are examples of genetically modified live vaccines. Because of such difficulties, the utilization of vaccination could be viewed as out of scope for such an operation(Hoelzer et al., 2018).

Phytochemicals

Phytochemicals are bioactive molecules originating from plants; phytochemicals or phytobiotics are added to animal feed to increase growth, productivity, etc.(Windisch et al., 2008). It has been proved that various phytochemical compounds show antimicrobial activity, such as kaempferol 3-O—L-(2″,3″-di-Z-p-coumaroyl)rhamnoside isolated from the leaves of the American sycamore showed strong antibacterial effectiveness against fish pathogenic bacteria (Mark T Hamann & Douglas L Rodenburg, 2015), hopeaphenol and vitisin isolated from roots of muscadine found and lycorine an alkaloid found in various plants showed highest antibacterial activity against *F. columnare*, the causative agent of columnaris disease in both cultured and wild freshwater fish (Schrader et al., 2018; Tan et al., 2011). Thus, treating medicinal plants with antibacterial activity might be a viable option in aquaculture, but it is challenging to translate in vitro investigations into in vivo applications in aquaculture. Other problems include concerns about the safety of phytochemicals produced from synthetic or semi-synthetic sources for feeding animals, inconsistent performance, enormous research and studies on dose and its pharmacodynamics, and loss in fiber content of animal feed fermentation. Furthermore, the yield of a substantial raw material is quite low(Nik Mohamad Nek Rahimi et al., 2022).

DISCUSSION

Antimicrobial resistance (AMR) poses a grave threat to human life, as it undermines the effectiveness of conventional antibiotics, leading to treatment failures and increased mortality rates. The emergence and spread of resistant microorganisms are fueled by various factors, including the extensive use of antibiotics in aquaculture and livestock farming. The misuse and overuse of antibiotics in these industries have contributed significantly to the development and dissemination of resistant strains, further exacerbating the AMR crisis.

Microorganisms possess inherent mechanisms that allow them to acquire and transfer resistance genes rapidly. This natural ability to develop resistance easily has discouraged pharmaceutical companies from investing in the development of new antibiotics. The economic feasibility and profitability of antibiotic research and development have been hampered by the high costs, lengthy timelines, and the likelihood of emerging resistance. As a result, the pipeline for new antibiotics has significantly dwindled in recent years, leading to a scarcity of effective treatment options against resistant pathogens.

To address the urgent need for alternative treatments and techniques, various approaches have been explored. Phage therapy, for instance, utilizes bacteriophages—viruses that infect bacteria—to target and eliminate specific pathogenic bacteria. In several studies, phage cocktails have shown superior efficacy compared to antibiotics, with the ability to reduce the risk of pathogenic invasion while sparing non-targeted commensal microbiota. However, challenges remain in terms of phage genome sequencing, formulation and stability of pharmacological formulations, and the potential for bacteria to develop resistance against phages.

Predatory bacteria have also emerged as potential alternatives to antibiotics. These bacteria possess the ability to prey on harmful microbes, offering a natural means of controlling pathogens. However, concerns exist regarding the potential for bacterial resistance to predatory bacteria and the incomplete eradication of the targeted pathogens. Further research is needed to fully understand and address these challenges.

Probiotic bacteria treatment involves the administration of beneficial bacteria to restore microbial balance and combat pathogens. The use of probiotics in aquaculture and livestock farming has shown promising results in preventing pathogen colonization. However, the presence of antibiotic-resistance genes in probiotics raises concerns about the potential spread of resistance to other microbes in the same environment. Strict monitoring and regulation are necessary to mitigate this risk and ensure the safe and responsible use of probiotics.

Antimicrobial peptides (AMPs) are another class of alternative treatments that show potential in combating resistant pathogens. These cationic peptides can interact with bacterial cell membranes, causing electrochemical changes, membrane degradation, and cell death. However, the high rate of failure in clinical studies and concerns about toxicity limit their application. Further research is needed to optimize their efficacy, safety, and delivery mechanisms.

Oligonucleotide aptamers, antibody-antibiotic conjugates, antisense/antigene

oligonucleotides, and artificial restriction nucleases offer promising avenues for combating AMR. However, challenges such as short half-lives, delivery mechanisms, and potential toxicities need to be addressed to ensure their successful clinical application. Efflux pump inhibitors, nanomaterials, vaccine development, and phytochemicals are also important areas of research. However, it is essential to acknowledge that while these alternatives hold promise in combating AMR, they cannot fully replace the need for new antibiotics. A comprehensive approach that combines the development of novel antibiotics, strict antimicrobial stewardship, infection prevention and control measures, and public education is necessary to address the global challenge of AMR effectively.

CONCLUSION

In conclusion, the emergence of antimicrobial resistance threatens human health worldwide, with aquaculture and livestock farming playing a significant role in its development and spread. The ease with which microorganisms acquire resistance, coupled with the decline in antibiotic development, necessitates the exploration of alternative treatments and techniques. Phage therapy, predatory bacteria, probiotic bacteria treatment, antimicrobial peptides, oligonucleotide aptamers, antibody-antibiotic conjugates, antisense/antigene oligonucleotides, artificial restriction nucleases, efflux pump inhibitors, nanomaterials, vaccine development, and phytochemicals offer potential solutions to combat AMR. However, these alternatives alone cannot fully fulfill the need for new antibiotics. A comprehensive approach that combines alternative treatments, antibiotic development, and stringent measures to reduce antibiotic use and promote responsible practices is crucial to mitigate the threat of AMR effectively.

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